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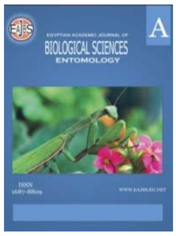


# A review About the Role of Hygienic Behavior as A defense Mechanism of Honey Bee against the Parasitic Mites and Diseases

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**ABSTRACT**

Hygienic behavior in honey bee (*Apis mellifera* L.) is measured by determining the rate at which the bee uncap and remove dead sealed broad. This collected review described the mechanism of hygienic against the parasitic mite (*Varroa destructor*), as well as, the brood diseases (Chalk Brood and American Foulbrood). Breeding hygienic disease resistant bees as an important option was determined by some investigators. However, heritability of hygienic behavior against *Varroa* was discussed by other investigators. Some researches were conducted to assess the performance of commercially bred honey bee queens sold as resistant to the parasitic mite, *V. destructor*. Physiological susceptibility and hygienic behavior affect chalk brood disease incidence in worker and drone larvae in honey bees (*A. mellifera*) took attention by others. Control of American Foulbrood disease in commercial apiaries through the use of queens selected for hygienic behavior was discussed in some articles. A comparison of the hygienic response of Africanized and European (*A. mellifera carnica*) honey bees to *Varroa*-infested brood in tropical areas was also investigated. An annotated list contains scope of study, authors and the studied area was concluded.

## INTRODUCTION

Hygienic behavior in honeybee (*Apis mellifera*,Hymenoptera: Apidae) has been shown to be an effective control mechanism against mites and diseases such as Chalk Brood (CB) and American Foulbrood (AFB) diseases. Chalk brood has proven to be problematic for the honey industry since it was identified. The earlier, Rothenbuhler (1964) investigated the genetic basis of hygienic behavior, proposing a two-gene model to explain the uncapping and removal of dead brood. His elegant experiment remains the textbook example of a behavioral genetic study. Although, this model has been challenged, it is still generally agreed that a small number of unlinked genes produce a large effect on hygienic behavior that hygienic alleles are recessive and are inherited in a Mendelian manner(Wilkes and Oldroyd, 2002).Honey bee hygienic behavior was investigated by several researchers all over the world, e.g.(Abdel-Wahab,1966,2001;Abdel-Rahman,2004; Amro,2009 and Abou-Shaara,2014) in Egypt, (Boecking,1992;Boecking and Drescher, 1992 and Boecking and Spivak,1999) in Germany, (Spivak, 1996, 1997; Spivak and Downey, 1998;Spivak and Gilliam 1998a,b;Spivak and Reuter 1998 a,b; 2008) in USA. Also, several publications dealt with this behavior in UK, Brazil, Argentina and some European countries.

## MATERIALS AND METHODS

**Sources of the review articles:**

An extensive review surveyof the hygienic behavior was recorded by the writer. Publications dealing with the mechanism of hygienic behavior, breeding resistant hygiene bees, heritability of hygiene honey bees' and breeding commercial hygiene queens were reviewed. Egyptian journals and bulletins in addition to conferences, approved theses and dissertations were the main source of information.

## RESULTS AND DISCUTION

**Data analysis:**

Data presented in Table (1) and illustrated in Figure (1) revealed that 89 researcheswere collected about hygienic behavior. The available researches were divided into five subjects in addition to the general studies. These subjects were arranged in descending order as follows: mechanism of hygienic behavior (38.20%); general studies (26.97%); comparative studies (20.22%); breeding resistant hygiene bees (7.87%); heritability of hygiene honey bees (4.49%) and breeding commercial hygiene queens(2.25%).

Data illustrated in Figure (2) revealed that researches published about the hygienic behavior were approved in 21 countries all over the world. The hygienic researches conducted in the United States of America (USA) alone confirmed 33.71%. However, researches conducted in the remaining countries confirmed 66.29%. Relatively concern of hygienic studies was observed in Germany, Brazil and Egypt. This subject could be reflecting the advance of honey bee industry in the developing countries. So, the collected hygienic researches can be classified to the following aspects:

**1. Mechanism of hygienic behavior:**

Study on an easy method of hygienic behavior evaluation in honey bee was conducted by Olszewski and Paleolog (2007) in Poland. They assessed the hygienic behavior by means of two test types: the traditional needle test and the removal rate of cardboard pieces.Sequential hygienic behavior in Carniolan honey bees (*Apis mellifera carnica*) was studied by Gramacho and Gonçalves (2009a) in Brazil. They reported that the most frequent sequence of events was: capped cell → punctured cell → brood partially removed → empty cell. A new model of three pairs of recessive genes (uncapping u1, u2 and remover was proposed in order to explain the genetic control of the hygienic behavior in *A. mellifera*. The authors recommend evaluating hygienic behavior 24 h after perforation and using a correction factor to compensate for control removal levels.

Relationship between dead pupa removal and season and productivity of honey bee (*A.mellifera*) colonies was studied by Guler and Toy (2013) in Turkey.

Table 1.A list of the hygienic behavior collected articles.

| Scope of study | Author(s) | Year | Country |
|---|---|---|---|
| **1. Mechanism of hygienic behavior** | Abdel-Wahab | 1996 | Egypt |
| | Abdel-Wahab | 2001 | Egypt |
| | Abou-Shaara | 2014 | Egypt |
| | Aumeier *et al.* | 1996 | Brazil |
| | Boecking | 1992 | Germany |
| | Boecking and Drescher | 1992 | Germany |
| | Boecking and Spivak | 1999 | Germany |
| | Casanova and Sierra | 1997 | Unknown |
| | Gilliam *et al.* | 1983 | USA |
| | Gramacho and Goncalves | 2001 | Brazil |
| | Ibrahim and Spivak | 2006 | USA |
| | Invernizzi | 2012 | Uruguay |
| | Johnson *et al.* | 2005 | USA |
| | Lawrence *et al.* | 2014 | Nigeria |
| | Masterman *et al.* | 2000 | USA |
| | Najafgholian *et al.* | 2011 | Iran |
| | Newton *et al.* | 1975 | USA |
| | Olszewski and Paleolog | 2007 | Poland |
| | Palacio *et al.* | 2010 | Argentina |
| | Paleolog | 2009 | Poland |
| | Pereira *et al.* | 2013 | Brazil |
| | Poklukar | 2000 | Slovenia |
| | Reuter and Spivak | 1998 | USA |
| | Rueppell *et al.* | 2010 | USA |
| | Spivak | 1996 | USA |
| | Spivak | 1997 | USA |
| | Spivak and Downey | 1998 | USA |
| | Spivak and Gilliam | 1998a | USA |
| | Spivak and Gilliam | 1998b | USA |
| | Spivak and Reuter | 1998a | USA |
| | Spivak and Reuter | 1998b | USA |
| | Spivak and Reuter | 2008 | USA |
| | Spivak *et al.* | 2001 | USA |
| | Vandame and Colin | 1999 | Unknown |
| **2. Breeding resistant hygiene bees** | Andere *et al.* | 2001b | Unknown |
| | Nelson *et al.* | 2003 | USA |
| | Pernal *et al.* | 2012 | Canada |
| | Rinderer *et al.* | 2010 | USA |
| | Spivak and Reuter | 2001a | USA |
| | Spivak and Reuter | 2001b | USA |
| | Wilkes and Oldroyd | 2002 | Unknown |
| **3. Heritability of hygiene honey bees** | Boecking *et al.* | 2000 | Germany |
| | Doleza. and Toth | 2013 | USA |
| | Rothenbuhler | 1964 | USA |
| | Stanimirovic *et al.* | 2008 | Serbia |

Table 1.(Contd.)

| Scope of study | Author(s) | Year | Country |
|---|---|---|---|
| **4. Breeding commercial hygiene queens** | Basualdo *et al.* | 2008 | Argentina |
| | Bryant | 2004 | USA |
| **5. Comparative studies on hygienic behavior** | Abdel-Rahman | 2004 | Egypt |
| | Amro | 2009 | Egypt |
| | Aumeier *et al.* | 2000 | Brazil |
| | Aumeier and Rosenkranz | 2001 | Germany |
| | Bak *et al.* | 2010 | Poland |
| | Balhareth *et al.* | 2012 | KSA |
| | Bar and Rosenkranz | 1992 | Unknown |
| | Danka *et al.* | 2013 | USA |
| | Gramacho and Goncalves | 1999 | Brazil |
| | Gramacho and Goncalves | 2009b | Brazil |
| | Kamal *et al.* | 2003 | Egypt |
| | Medina *et al.* | 2009 | UK |
| | Morais*et al.* | 2010 | Brazil |
| | Palacio *et al.* | 2001 | Argentina |
| | Rodrigues *et al.* | 1996 | Unknown |
| | Spivak and Reuter | 1998c | USA |
| | Spivak and Reuter | 2001c | USA |
| | Stanimirovic *et al.* | 2002 | Unknown |
| **6. General studies** | Adjlane *et al.* | 2011 | Algeria |
| | Adjlane and Haddad | 2014 | Algeria |
| | Aleksandar *et al.* | 2014 | Europe |
| | Al Fattah *et al.* | 2012 | Egypt |
| | Andere*et al.* | 2001a | Unknown |
| | Andino. and Hunt | 2011 | USA |
| | Aumeier | 2001 | Germany |
| | Baracchi *et al.* | 2012 | Italy |
| | De Guzman *et al.* | 2002 | Russia |
| | Goode *et al.* | 2006 | USA |
| | Gramacho and Goncalves | 2009a | Brazil |
| | Guler and Toy | 2013 | Turkey |
| | Hegić and Bubalo | 2006 | Poland |
| | Locke and Fries | 2011 | Sweden |
| | Nicodemo *et al.* | 2013 | Brazil |
| | Nunes-Silva *et al.* | 2009 | Brazil |
| | Palacio and Bedascarrasbure | 2001 | Argentina |
| | Pe´rez-Sato *et al.* | 2009 | UK |
| | Rosenkranz *et al.* | 1997 | Germany |
| | Schöning *et al.* | 2012 | Germany |
| | Spivak*et al.* | 2003 | USA |
| | Spivak and Reuter | 1998c | USA |
| | Stanimirovic | 2003 | Unknown |
| | Waite *et al.* | 2003 | UK |

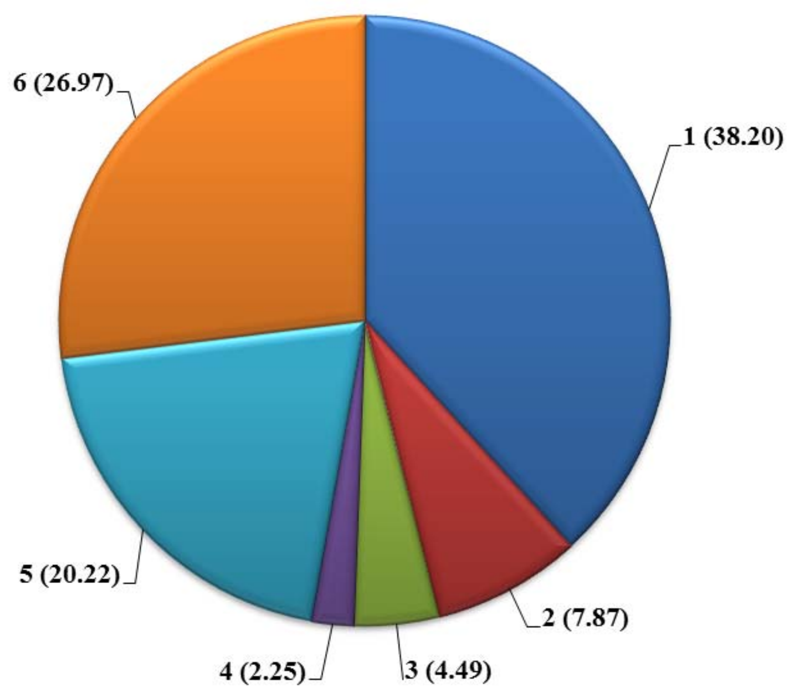


**Fig. 1- Scope of study percentages**

1. Mechanism of hygienic behavior 2. Breeding resistant hygiene bees
3. Heritability of hygiene honey bees 4. Breeding commercial hygiene queens
5. Comparative studies on hygienic behavior 6. General studies

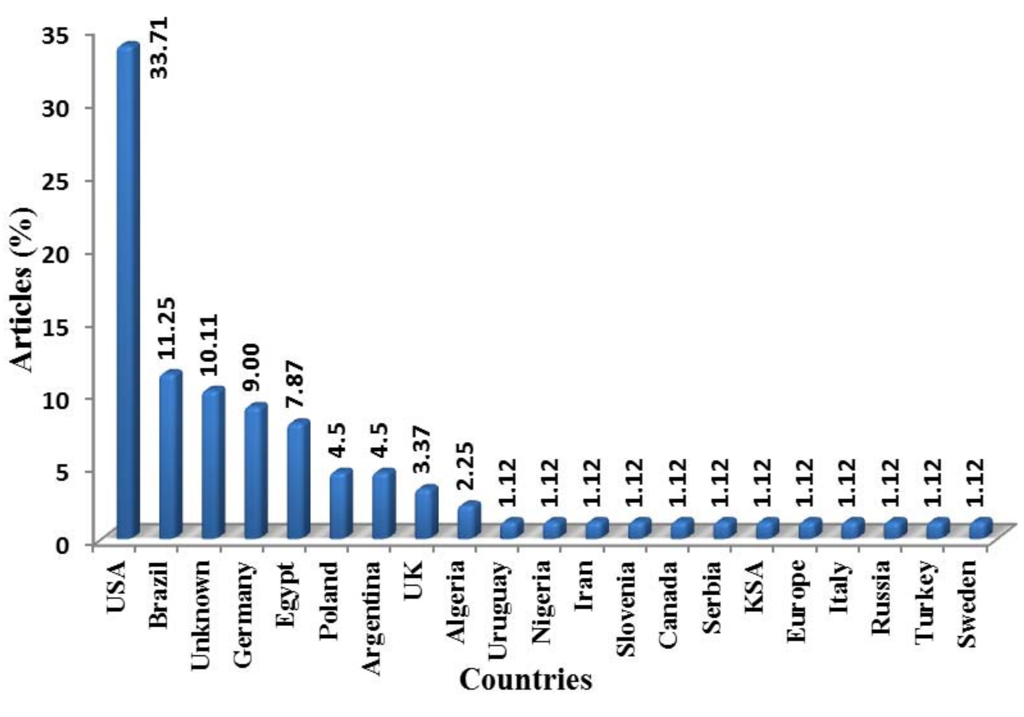


**Fig. 2- Hygienic behavior collected articles % for countries**

The liquid nitrogen technique was used. There were significant positive correlations between dead pupae removal and honey yield. Although hygienic behavior has positive effects on many characters relating to productivity in untreated breeding populations, it is affected by many biotic and abiotic factors. This behavior decreases with many stressful conditions (wasps, *Merops* sp., predators, honey harvesting) and increases with colony strength during the season.

Hygienic behavioral mechanism of resistance to diseases and parasites in West African honey bee colonies *A. mellifera adansonii*, was studied by Lawrence *et al.* (2014) in Nigeria. They grouped colonies into two categories: healthy colonies (HC) when they are free from parasites and brood diseases and infected colonies (IC) when they show the presence of parasites and brood diseases. Hygienic test was carried out using pin to kill some brood in both HC and IC; the numbers of dead broods uncapped and removed within 24 and 48hours were noted. Pin-killed brood assay revealed removal of dead brood within 24 and 48 hours. In *A .mellifera adansonii*, uncapping and removal of dead brood in hygienic colonies is a mechanism of resistance and in infected colonies in addition to resistance, it reduces the spread of diseases and parasitic infections.

**2. Breeding resistant hygiene bees:**

Resistance to American Foulbrood disease by honey bee colonies *A.mellifera* bred for hygienic behavior was studied by Spivak and Reuter (2001a). Approximately, 39% of the hygienic colonies developed clinical symptoms of the disease but five of these recovered (had no visible symptoms) leaving two colonies (11%) with clinical symptoms. In contrast 33% of the hygienic colonies developed clinical symptoms of Chalk Brood after they were challenged with American Foulbrood, but all recovered. The diseased non-hygienic colonies produced significantly less honey than the hygienic colonies.

In USA, honey bee (*A. mellifera*) colonies bred for hygienic behavior were tested in a large field trial by Spivak and Reuter (2001b) to determine if they were able to resist the parasitic mite *Varroa destructor* better than unselected colonies of “Starline” stock. Colonies bred for hygienic behavior are able to detect, uncap, and remove experimentally infested brood from the nest. Although, the extent to which the behavior actually reduces the overall mite-load in untreated, naturally infested colonies needed further verification. The results indicated that hygienic colonies with queens mated naturally to unselected drones had significantly fewer mites on adult bees and within worker brood cells than Star line colonies for up to 1 yr. without treatment in a commercial, migratory beekeeping operation. Hygienic colonies actively defended themselves against the mites when mite levels were relatively low.
Breeding for hygienic behavior in honeybees (*A.mellifera*) using free-mated nucleus colonies was studied by Pernal *et al.* (2012) in Canada. Gains in hygienic behavioral based on maternal selection of queens were evaluated among four commercial beekeeping operations in northern Alberta, Canada over four generations. Their work implies that year-to-year comparisons of breeding colonies may not be an accurate predictor of selection gains within honeybee populations and that honey producers in this region may need to focus selection within elite breeding populations.

**3. Heritability of hygiene honey bees:**

Heritability of hygienic behavior in honey bees against *Varroa*-species was studied by Boecking *et al*. (2000) in Germany. In this study the heritability of the hygienic behavior of *A.mellifera*bees was estimated on the basis of the mother daughter regression. The heritability for the hygienic behavior towards *V. destructor* infested brood cells was h2.0.18 (+0.27) and h2.0.36 (+0.30) for the hygienic behavior towards dead brood cells. The repeatability was likewise higher for the pin-killed brood assay (W.0.46) compared with the assay using living mites-infested brood cells (W.0.24).

Experimental backcross colonies were produced by Wilkes and Oldroyd (2002) from an inbred hygienic line and an inbred no hygienic line, both provided by Dr. Marla Spivak, University of Minnesota. These backcross colonies were assessed for hygienic behavior using a standard assay. Molecular techniques, linkage mapping and QTL analysis then were employed to determine how many loci directly influence hygienic behavior and the relative level of influence and location of each locus within the genome of *A. mellifera*.

Breeding grey *A.mellifera carnica* queens for hygienic behavior was evaluated by Stanimirovi *et al*. (2008) in Serbia. The heritability values for hygienic behavior were h2=0.63±0.02 in relationship between daughter queens (F1s) and breeder mother queens (Ps), h2=0.45±0.01 in relationship between grand-daughter queens (F2s) and Ps and h2=0.44±0.02 in relationship between F2s and F1s. These results show that the expression of hygienic behavior through selective breeding could be enhanced and the best results could be achieved in the F1 generation.

**4. Breeding commercial hygiene queens:**

Bryant (2004) research was conducted to assess the performance of commercially bred honey bee queens sold as resistant to the parasitic mite, *V. destructor* in USA. The study's objectives were to: 1) compare honey and pollen stores and *V. destructor* infestation in colonies established with hybrid Russian, SMR, and control queens, 2) determine levels of hygienic behavior and mite non-reproduction in the same colonies, and 3) determine the relationship between juvenile hormone III in honey bee larvae and *V. destructor* reproduction.

Spivak and Reuter(2008) bred honey bees for resistance to diseases and *V. destructor* since 1994 with the goal of reducing, or eliminating, the use of antibiotics and pesticides by beekeepers. Hygienic bees resist these diseases and mites by detecting diseased or mite-infested brood and quickly removing the infected brood from the nest. Beekeepers considered that breeding for this trait might compromise honey production or make their colonies more defensive. This study showed that in addition to helping the bees resist AFB, chalk brood and *Varroa*, the Hygienic line is a good honey producer, and is gentle and easy to manage. Today, queens from the "Hygienic" line can be purchased nationally and the feedback received tells that the line is well respected by beekeepers. The rapid removal of freeze-killed brood is correlated with the removal of diseased and mite-parasitized brood. It is then easy to raise queens from colonies that are hygienic.

Control of American foulbrood disease in commercial apiaries through the use of queens selected for hygienic behavior was evaluated by Basualdo *et al*., (2008) in Argentine. Hives positive for AFB were isolated from the apiary and the queens of the remaining hives replaced by others selected for hygienic behavior. The prevalence of AFB was then recorded by monitoring visual signs of the disease, and via the isolation of bacterium *Paenibacillus larvae* spores. The hives showed no clinical signs of AFB although honey samples testing showed 50% to harbor *P. larvae* spores after one year. This percentage decreased to 26% by the end of the study.

**5. Comparative studies on hygienic behavior**

A comparison of the hygienic response of Africanized and European (*A.mellifera carnica*) honey bees to *Varroa*-infested brood in tropical Brazil was determined by Aumeier *et al.* (2000).The hygienic response varied between

Africanized and Carniolan colonies, but this difference was significant only in one year, during which Africanized honey bees removed a significantly greater proportion of *Varroa* mites than European honey bees. They found variation between colonies in the reaction towards *Varroa*-infested brood of Africanized honey bees compared to Carniolans. The overall similar response of the two bee types indicates that hygienic behavior is not a key factor in the tolerance to Varroosis of Africanized bees in Brazil.

A comparative study of the hygienic behavior of Carniolan and Africanized honey bees directed towards grouped versus isolated dead brood cells was studied by Gramacho and Gonçalves (2009b) in Brazil. Isolated cells were more frequently cleaned than grouped cells, though variance analysis showed no significant difference. Carniolan bees also were somewhat, though not significantly more hygienic than Africanized honey bees. Results conclude that honey bees can detect and remove both isolated and grouped dead brood.

Comparison of Hygienic and Grooming behaviors of indigenous and exotic honeybee (*A.mellifera*) races in central Saudi Arabia was studied by Balhareth *et al.* (2012). Time consumed to detect and remove dead brood in colonies of the two races was variable i.e., native race differentiated and removed dead brood faster than exotic one. Deformed *Varroa* mites, as a result of grooming behavior, were significantly higher in colonies of native race than those of exotic one. The remarkable defensive behavior exhibited by the native race, compared to that of exotic one, may be due to its different genetic structure, as well as, its compatibility with local environmental conditions.

The susceptibility of bee larvae to *Ascosphaera apis* infestation and the hygienic behavior of worker bees in relation to *A. apis* infected and freeze-killed brood were evaluated in three races of bees: *A.mellifera carnica, A.mellifera caucasica*, and *A.mellifera mellifera* by Panasiuk*et al.*(2014) in Poland. Distinct variations were recorded between the tested races.

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## ARABIC SUMMERY

**دراسة مرجعية حول دور السلوك الصحي لدى نحل العسل كميكانيكية دفاع ضد الحلم المتطفل وأمراض النحل**

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سلوك التخلص من الحضنة المصابة في نحل العسل يقاس بتعريف المعدل الذي يقوم به النحل لكشف غطاء العيون السداسية و أزالة الحضنة الميتة. و قد قامت هذه الدراسة بوصف ميكانيكية التخلص من الحلم المتطفل (الفاروا) و من الحضنة المصابة بمرض الحضنة الطباشيري و مرض الحضنة الأمريكي. كما أمكن التعرف علي طرق تربية نحل مقاوم لأمراض النحل. أيضا أظهرت المراجع الطرق التي قام بها بعض الباحثين لأمكانية توريث هذا السلوك لمقاومة الفاروا. بعض الأبحاث المتحصل عليها أظهرت أمكانية تربية ملكات تجارية تتصف بتنظيف الخلايا من الفاروا.كماأظهرت بعض الأبحاث وجه المقارنة بين أستجابة النحل الأفريقي و النحل الكرنيولي في القدرة علي مقاومة الفاروا في المناطق الحارة. هذا و قد أدرجت قائمه بالمراجع و أسماء الباحثين و المناطق و البلدان التي أهتمت بدراسة هذا السلوك علي مستوي العالم .

**الكلمات الدالة:** سلوك التخلص من الحضنة المصابة ، نحل العسل ، الفاروا و أمراض النحل .